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ORIGINAL RESEARCH

Gankyrin Promotes Tumor Suppressor Protein Degradation to Drive Hepatocyte Proliferation

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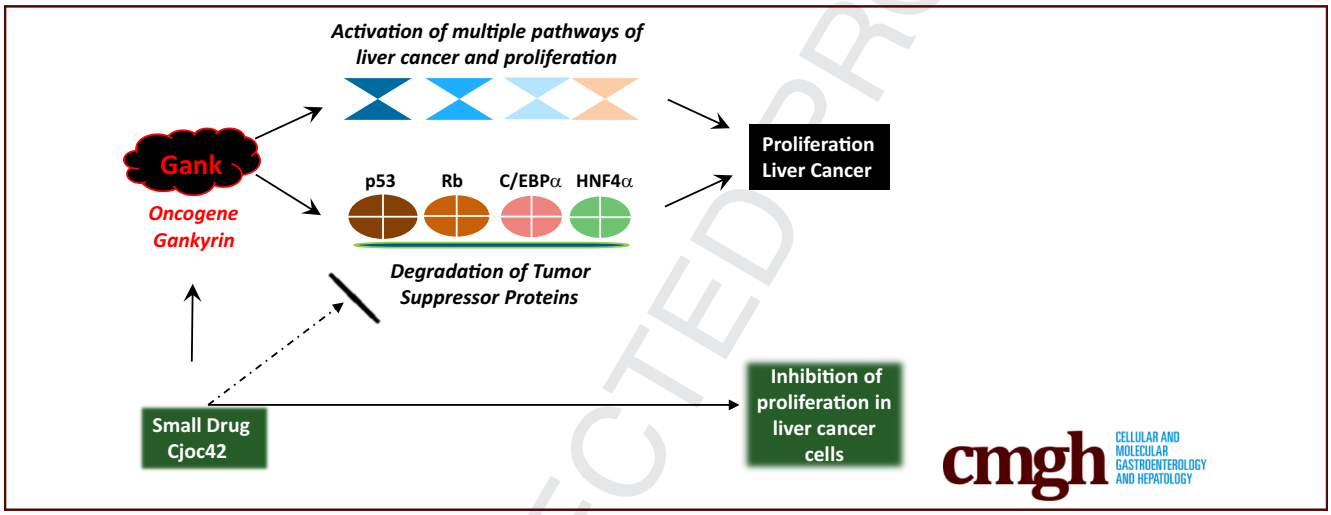

SUMMARY

The mechanisms by which gankyrin promotes hepatic proliferation are not known. This study shows that gankyrin promotes proteosomal degradation of tumor-suppressor proteins. Gankyrin deletion restored tumor-suppressor protein expression and delayed regenerative hepatocyte proliferation in vivo. Furthermore, proteasome inhibition limited growth of human- and mouse-derived liver cancer cell lines in vitro.

BACKGROUND & AIMS: Uncontrolled liver proliferation is a key characteristic of liver cancer; however, the mechanisms by which this occurs are not well understood. Elucidation of these mechanisms is necessary for the development of better therapy. The oncogene Gankyrin (Gank) is overexpressed in both hepatocellular carcinoma and hepatoblastoma. The aim of this work was to determine the role of Gank in liver proliferation and elucidate the mechanism by which Gank promotes liver proliferation.

METHODS: We generated Gank liver-specific knock-out (GLKO) mice and examined liver biology and proliferation after surgical resection and liver injury.

RESULTS: Global profiling of gene expression in GLKO mice showed significant changes in pathways involved in liver cancer and proliferation. Investigations of liver proliferation after partial hepatectomy and CCl_4 treatment showed that GLKO mice have dramatically inhibited proliferation of hepatocytes at early stages after surgery and injury. In control LoxP mice, liver proliferation was characterized by Gank-mediated reduction of tumor-suppressor proteins (TSPs). The failure of GLKO hepatocytes to proliferate is associated with a lack of down-regulation of these proteins. Surprisingly, we found that hepatic progenitor cells of GLKO mice start proliferation at later stages and restore the original size of the liver at 14 days after partial hepatectomy. To examine the proliferative activities of Gank in cancer cells, we used a small molecule, cjoc42, to inhibit interactions of Gank with the 26S proteasome. These studies showed that Gank triggers degradation of TSPs and that cjoc42-mediated inhibition of Gank increases levels of TSPs and inhibits proliferation of cancer cells.

CONCLUSIONS: These studies show that Gank promotes hepatocyte proliferation by elimination of TSPs. This work provides background for the development of Gank-mediated therapy for the treatment of liver cancer. RNA sequencing data can be accessed in the NCBI Gene Expression Omnibus: GSE104395. *(Cell Mol Gastroenterol Hepatol 2018;■:■–■; https://doi.org/10.1016/j.jcmgh.2018.05.007)*

Keywords: Liver; Proliferation; Cancer; Tumor Suppressor Proteins; Progenitor Cells.

The development of liver cancer is associated with multiple alterations in cellular function and gene expression.[1] One of the main hallmarks of liver cancer is uncontrolled proliferation, which is owing in part to damage of pathways essential to cell-cycle control. In addition, regulation in the coordinated expression of oncogenes and tumor-suppressor proteins (TSPs) is vital to tumor proliferation. One of the key oncogenes and promoters of liver proliferation is a small subunit of 26S proteasome, Gankyrin (Gank). This non–adenosine triphosphatase subunit of the ubiquitin proteasome system (UPS) is a notorious oncogene expressed in several cancer types, including hepatocellular carcinoma (HCC), in which it was first discovered.[2–4] In agreement with these observations, Gank has been identified as the driver oncogene in the early development of liver cancer through chemical models as well as age-dependent hepatic tumorigenesis.[2–7]

Gank promotes the development of HCC through several mechanisms, including the neutralization of TSPs. TSPs are the main proteins that support the quiescent status of the liver, and it has been shown that the activities of more than 20 different TSPs are lost in HCC because of mutations or hypermethylation of their promoters.[8] In addition, the elimination of TSPs by Gank is essential to carcinogenesis.[4] Specifically, Gank leads to the neutralization of essential TSPs such as p53, through stabilization of MDM2 ligase and subsequent enhanced ubiquitination, and Rb, by direct interaction, both of which trigger UPS-mediated degradation.[2,3,9] Studies of liver cancer have identified 2 additional targets of Gank: CCAAT/enhancer binding protein α (C/EBPα) and hepatocyte nuclear factor 4α (HNF4α).[6,7,9] C/EBPα belongs to the C/EBP family of proteins, bZIP proteins, which contain basic region and leucine zipper regions.[10] C/EBPα has been shown to be a strong inhibitor of proliferation and a strong TSP.[4,6,10,11] In fact, several recent reports with activation of the C/EBPα gene in animal models of liver carcinogenesis showed that its activation leads to inhibition of liver proliferation and carcinogenesis as well as normalization of liver function.[12–14] HNF4α is also a strong TSP and expression of this protein correlates with the epithelial–mesenchymal transition involved in metastatic tumor formation.[15] It also has been shown that deletion of HNF4α promotes hepatocyte proliferation and diethylnitrosamine (DEN)-induced liver cancer.[16] In addition to these known TSPs, recent studies have identified RNA CUG triplet repeat binding protein 1 (CUGBP1) as a tumor suppressor, whose activity depends on phosphorylation/dephosphorylation at serine 302.[17] Generation of CUGBP1-S302A KI mice showed that this TSP protects the liver from the development of cancer and that during liver carcinogenesis, Gank eliminates this isoform of CUGBP1.[17] In agreement with this, livers of CUGBP1 knock-out mice show a molecular signature of hepatoblastoma and express increased levels of stem cell markers and reduced levels of markers of hepatocytes.[9]

Increasing evidence has shown how Gank is responsible for the activation of additional pathways critical to liver cancer. For example, in addition to its effect on TSPs, Gank also stabilizes the stem cell marker Oct4 through elimination of WWP2, the ubiquitin ligase that normally marks Oct4 for degradation.[18] To promote uncontrolled proliferation, Gank also binds to D-type kinase, cdk4, and replaces $p16^{INK4a}$ from cdk4, leading to the activation of cdk4 and cell-cycle progression.[2] In addition, Gank increases levels of oncogene Nrf2 by the elimination of Keap1 ligase, which triggers degradation of Nrf2.[19]

Regulation of activities of Gank in the liver is quite complicated. In the quiescent liver, farnesoid X receptor (FXR) partially represses Gank, however, with DEN-mediated carcinogenesis, there is a reduction of FXR, activation of Gank, and subsequent activation of the cascade of Gank-dependent pathways including loss of TSPs.[7] Our recent article showed that activation of FXR by GW4064 inhibits the development of liver cancer and that the FXR–Gank axis is involved in the development of pediatric liver cancer.[9] In agreement with these findings, a recent report showed that DEN-mediated liver cancer is reduced significantly in mice with liver-specific deletion of Gank.[20]

In this study, we examined the proliferative activities of Gank in recently generated liver-specific Gank liver-specific knock-out (LKO) mice. By using 2 models of liver proliferation/regeneration, partial hepatectomy (PH), and CCl_4 treatments, we obtained evidence showing that Gank promotes liver proliferation via direct interaction and elimination of at least 5 TSPs. We also found that inhibition of Gank by a small drug, cjoc42, inhibits proliferation of liver cancer by blocking the Gank–TSPs axis, suggesting that cjoc42 might be considered a novel therapy approach.

Methods

Animals

Experiments with animals were approved by the Institutional Animal Care and Use Committee at Cincinnati Children's Hospital (protocol IACUC2014-0042). A Gank LKO (GLKO) mouse model was created using the Cre–Lox system. Mice expressing the Cre recombinase protein driven by the albumin promoter were crossed with mice that had LoxP sequences flanking exons 2–4 of the Gank gene. The resulting offspring had the Gank gene excised only in cells expressing albumin.

Histology

Liver tissue was taken from the left lobe and fixed in 4% formaldehyde. Mice were injected intraperitoneally with

Abbreviations used in this paper: **BrdU, bromodeoxyuridine; cDNA, complementary DNA; C/EBP, CCAAT/enhancer binding protein; Co-IP, co-immunoprecipitation; CUGBP1, CUG triplet repeat binding protein 1; DEN, diethylnitrosamine; FXR, farnesoid X receptor; Gank, Gankyrin; HCC, hepatocellular carcinoma; GLKO, Gankyrin liver-specific knock-out; HNF4α, hepatocyte nuclear factor 4α; LKO, liver-specific knock-out; mRNA, messenger RNA; Opn, osteopontin; PCNA, proliferating cell nuclear antigen; PH, partial hepatectomy; Rb, _____; RT-PCR, reverse-transcriptase polymerase chain reaction; TSP, tumor-suppressor protein; 2D, 2-dimensional; UPS, ubiquitin proteasome system; WT, wild-type.**

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